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As a Guide in the Treatment of Bacterial Infection

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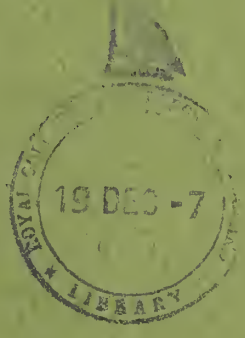

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THE VALUE OF THE OPSONIC INDEX
AS A GUIDE IN THE TREATMENT OF BACTERIAL INFECTION.*

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HISTORICAL.—As far back as 1858 Haeckel had observed that particles of indigo injected into the veins of certain molluscs could shortly afterwards be found in the blood cells of the animal. However, the significance of this and other similar observations was not appreciated, until Metchnikoff in 1883 called attention to their bearing on infection and immunity. The outcome of his investigations was the establishment of the well-known doctrine of *phagocytosis,* the principle of which is that the leucocytes attack and destroy the micro-organisms which have invaded the tissues. Metchnikoff's theory of immunity found but little acceptance, the more so since the studies of Nuttall, Pfeiffer, Behring, Ehrlich and others demonstrated a great variety of protective substances present in the serum of immune animals. As time went on Metchnikoff also realized that the serum constituents played an important part in the destruction of bacteria, but he interpreted this action as being a stimulation of the leucocytes, and he spoke of these substances as *stimulins.*

In 1903 A. E. Wright, of England, discovered certain constituents in blood serum which acted on bacteria and rendered them more easily taken up by the phagocytic cells. He called this substance *opsonin* and showed that it was present in normal serum and in the serum of infected individuals, though in the latter, as will be discussed directly, it varied considerably from the average normal content. By means of absorption tests, modeled after those of Ehrlich and Morgenroth, he showed that the opsonin has a specific affinity for the bacteria and none for the leucocytes. The opsonins for staphylococcus prepare only staphylococci, those for tubercle only tubercle bacilli, etc. If perfectly fresh, washed, normal leucocytes are mixed with a bacterial emulsion, phagocytosis is very slow, so slow in fact that at the end of fifteen to twenty minutes at 37 degrees C. the cells may still be empty. Under the influence of a serum containing opsonin the same leucocytes would have loaded themselves with bacteria, as can be seen by examining such a parallel control. It may be added that the leucocytes are believed by Metchnikoff to be the source of the opsonins. This author shows that the leucocytes act as phagocytes even without free opsonin, only it takes them longer to do it. The work of Hektoen seems to have clearly established that opsonins are distinct antibodies and not identical with bacteriolytic immune bodies. In the further study of these opsonins Wright developed the idea that they were highly important in combating a number of bacterial infections, and soon published observations on the opsonins in tubercle and staphylococcus infections. His observations showed that inoculations of the corresponding bacteria produced marked

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changes in the opsonic content of the infected individual, and that it was possible to estimate accurately the immunizing effect of such inoculations. At the present time he has correlated all these observations and built up a system of treating bacterial infections by means of active immunization controlled by opsonic measurements.

TECHNIQUE. Wright's technique of measuring the opsonic power is a slight modification of the Leishman[1] method and is as follows: An emulsion of fresh human leucocytes is made by dropping twenty drops of blood from a finger prick into 20 cc. normal salt solution containing one per cent. sodium citrate. The mixture is centrifuged, the supernatant clear fluid removed and the upper layers of the sedimented blood cells transferred by means of a fine pipette to 10 cc. normal salt solution. After centrifuging this second mixture the supernatant fluid is pipetted off and the remaining suspension used for the opsonic tests. Such a "leucocyte emulsion" of course still contains an enormous number of red blood cells; the proportion of leucocytes, however, is greater than in the original blood. One volume of this emulsion is mixed with one volume of the bacterial suspension to be tested and with one volume of the serum. This is best accomplished by means of a pipette made by drawing a capillary end to a glass tube. With a wax-pencil mark about three-quarter inch from the end, it is easy to suck up one such volume of each of the fluids, allowing a tiny air bubble to intervene between the different fluids. All these are now expelled on a slide and thoroughly mixed by drawing back and forth into the pipette. Then the mixture is sucked into the pipette, the end sealed in a flame, and the whole put into the incubator at 37° C. The identical test is made using a normal serum in place of the serum to be tested. Both tubes are allowed to incubate fifteen minutes and then examined by means of smear preparations on slides spread and stained in the usual way. The degree of phagocytosis is then determined in each by counting a consecutive series of fifty leucocytes and finding the average number of bacteria ingested per leucocyte. This number for the serum to be tested is divided by the number obtained with the normal serum and the result regarded as the *opsonic index* of the serum in question. The presence of a high index Wright regards as indicative of increased resistance. He further states that fluctuations of the opsonic index in normal healthy individuals is not more than from 0.8 to 1.2 and that an index below 0.8 is therefore diagnostic of the presence of an infection with the organism tested. It should be stated that for the normal control Wright usually employs a mixture of several normal sera, thus seeking to secure a fair normal average.

BACTERIAL INOCULATIONS. Wright's method of treating bacterial infections is based on the following premises: In localized infections the infected body absorbs but small amounts of bacterial substances or antigens. In consequence of this the amount of active immunity developed is but slight. Localized infections therefore tend to run a chronic course. The logical method of effecting a cure in these cases is to actively immunize the body with the invading organism.[1] In a number of infections, notably those of staphylococcus, streptococcus, and tubercle the degree of immunity is measured accurately, according to Wright, by the opsonic power of the blood serum, i. e., by the opsonic index. He believes it is almost impossible, by mere clinical observation,

[1]Leishman, British Medical Journal, January, 1902.

[1]It is difficult to understand on what grounds Wright uses the bacterial inoculations in systemic infections such as a malignant streptococcus endocarditis with organisms in the blood. According to the above premises a systemic infection should already have flooded the body with so much antigen that an additional inoculation would be contra-indicated.

to determine whether or not the bacterial inoculations are producing increased immunity; that this is comparatively easy by means of opsonic measurements.

The bacterial inoculations consist of suspensions of agar cultures in normal salt solution. These suspensions are heated to 55 degrees or 60 degrees C. for twenty minutes in order to kill the organisms, and then receive small additions of carbolic acid or lysol as preservatives. Cultural tests are made to ensure sterility and the "vaccine" counted so as to determine the dose. This is readily accomplished by means of a small capillary pipette such as is used for the opsonic test. A wax-pencil mark is made on the capillary tube and then one such volume of the vaccine to be tested, and one volume of blood taken from a fresh finger prick (an air bubble intervening) are sucked into the pipette. The two fluids are thoroughly mixed by sucking back and forth on a slide and the mixture then spread on a slide in the ordinary way for blood smears. After staining it is comparatively easy to ascertain, by counting through a ruled ocular, the proportion of bacteria to red blood cells. The latter being regarded as 5,000 million per cc., it needs but a simple calculation to give the number of bacteria per cc. So far as dosage is concerned one employs from 300 to 500 million in the case of staphylococcus, from 50 to 100 million in the case of streptococcus, etc.

ORIGINAL INVESTIGATIONS.—The experiments here reported were directed by Dr. W. H. Park and carried out by a number of the workers, including the writer, at the Research Laboratory of the N. Y. City Health Department.

In counting a consecutive series of 50 or 60 leucocytes, as is done by Wright and his pupils, one cannot help being struck by the enormous differences in phagocytic power exhibited by the different leucocytes. Thus, using a perfectly homogeneous emulsion of staphylococcus with which the average of several hundred cells will be 5 or 6 bacteria to the leucocyte, it is not at all uncommon to find a number of cells having as high as 20 or 25 bacteria, and cells containing no bacteria at all. The staining characteristics of these cells give no indication of any difference, both are apparently perfectly normal leucocytes. The difference in the total count of 50 cells when several of these loaded cells are or are not encountered is obvious. From the first, therefore, it was felt that the counting of merely 50 leucocytes was subject to too large fluctuations to be really accurate, and all the work from which the conclusions in this paper are drawn was therefore done with counts of from 150 to 200 leucocytes. The following table gives an idea of the divergence in the several fifties occasionally encountered when counting 200 cells:

	EXAMPLE 1.		EXAMPLE 2.		EXAMPLE 3.	
	Total in Fifty.	Per Cell.	Total in Fifty.	Per Cell.	Total in Fifty.	Per Cell.
First fifty.....	94	1.88	100	2.00	78	1.56
Second fifty.....	84	1.68	101	2.02	46	.92
Third fifty.....	67	1.34	115	2.30	95	1.90
Fourth fifty.....	57	1.14	138	2.76	72	1.44
Average for the 200 cells		1.51		2.30		1.45

The same is shown in the following table in which 350 cells were counted:

First fifty.....	2.82	aver. No.	bacteria per cell
Second fifty...	1.96	"	" "
Third fifty....	2.06	"	" "
Fourth fifty...	2.38	"	" "
Fifth fifty....	2.48	"	" "
Sixth fifty....	2.70	"	" "
Seventh fifty..	1.88	"	" "

2.32 average of 350 cells.

We have many protocols, to be sure, in which these divergences are not so

marked. It is doubtful, though, whether in a long series of cases the difference between an average based on 50 cells and one based on 200 cells can be made much less than 20 per cent. We would, therefore, say that a count of 50 cells may give a rough indication of the count; 100 cells probably a pretty fair approximation; while a count of 200 cells usually indicates very closely the conditions prevailing in that particular test.

Attention was directed next to the opsonic indices of apparently normal individuals, and it was soon realized that, at least so far as tubercle, staphylococcus and streptococcus are concerned, the indices often differ very widely in different individuals. This is shown by the following table which shows the opsonic index in two normal individuals day by day. In order to facilitate comparison the same person has always been regarded as 1. The method of testing and the entire technique was that developed by Wright and demonstrated in this city by him and his asociate, Dr. Ross. The average counts, however, are based on 200 leucocytes instead of but 50, as is usual with Wright. The organism tested was a staphylococcus aureus.

TABLE I.

Fluctuations in Opsonic Power in Two Normal Sera.

DATE.	RATIO.	DATE.	RATIO.
Dec. 19	1—1.57	Jan. 6	not done
20	1—1.32	7	1—0.90
21	1—0.82	8	not done
22	1—0.76	9	1—1.40
23	not done	10	not done
24	not done	11	1—1.65
25	not done	12	1—0.92
26	1—1.16	13	not done
27	not done	14	1—1.06
28	1—0.92	15	not done
29	1—0.82	16	1—1.19
30	not done	17	1—0.91
31	1—1.83		
Jan. 1	not done		
2	1—1.18		
3	1—1.18		
4	1—0.90		
5	1—2.50		

With fluctuations as great as those here shown it seems absolutely unscientific to take the average of two or three such "normal" counts with which to compare the test serum. Other observers have had similar variations, though in some instances, at least, they have not hesitated to take the average of even widely different counts. The following is another example of such variations, three normal persons being used as a control. This is also a staphylococcus case:

Average count for patient's serum				11.97
" " " control			A	13.40
" " " "			B	9.03
" " " "			C	6.14

What the patient's opsonic index in this case really was it is hard to say.

There is still another source of discordant results as is shown by the following curious phenomenon. Instead of testing a given blood but once, two, three, four or more tubes of it are tested at the same time and under identical conditions so far as we can determine. The same is done with the control serum. The test can be made either by collecting a number of tubes of blood from the same individual, or by collecting but one tube and then making duplicate or triplicate tests on this specimen. The following table gives the results of one of many tests of this kind. The organism used was a staphylococcus aureus and the number of cells counted for each tube was 150.

PATIENT'S SERUM.

Tube 1.	Average per leucocyte	.....	2.15
" 2.	" " "	.....	2.47
" 3.	" " "	.....	2.70
" 4.	" " "	.....	2.24

CONTROL SERUM.

Tube 1.	Average per leucocyte	.....	2.04
" 2.	" " "	.....	1.87
" 3.	" " "	.....	1.85

From these figures it is possible to calculate indices ranging from 1.05 to 1.46.

These results are so surprising that we have repeated such tests again and again. In testing a given serum with,

say, six or seven tubes one often finds many of the tubes giving very similar results. As a rule, however, one or two of the tubes will give counts far away from the average.

Other workers in this city have been kind enough to co-operate with us in making tests of this kind; every one of these has reported results similar to the preceding. The following figures were obtained by an experienced opsonologist on numbered sera sent him by us. (Tests refer to tubercle.)

Serum No.	1	2.1
"	2	2.1
"	3	3.1
"	4	3.3
"	5	1.6
"	6	2.35
"	7	2.16
"	8	2.98
"	9	2.90
"	10	1.40

The brackets indicate the sera which this observer regarded as identical. As a matter of fact, however, the first six sera were from one normal individual; the next two (Nos. 7 and 8) were from another normal individual; and No. 9 and 10 were from two patients. In this case one patient's index can be figured as from 0.55 to 1.13, and the other as from 0.42 to 0.87.

Reviewing the data presented above it is seen that the opsonic index of a given patient as it is usually obtained by Wright's method is far from accurate. The most that one is warranted in saying is that marked variations from the "normal" opsonic index probably indicate that the serum in question is "high" or "low" as the case may be. Variations of 0.2 or 0.3 or even of 0.4 are of very little value *provided one test only is made and only* 50 *cells counted.* To illustrate, an index of 2.5 undoubtedly indicates an increased opsonic power, an index of 0.4 or 0.5 a decreased opsonic power. But a change from 2.5 to 2.0 means very little, as also does a change from 0.5 to 0.8.

It is reasonable to assume that indices obtained by making two or more opsonic tests of the same serum and then counting 100 or 150 leucocytes represents very nearly the true condition of the individual's serum. The amount of labor involved in such a test is, of course, considerable and cannot ordinarily be expended in a given case. It was felt, however, that only in this way could one determine the accuracy of the principles underlying Wright's method of treatment.

According to Wright the inoculation of a bacterial culture is followed almost always by a period in which the opsonic index is lowered; this he calls the negative phase. Then, depending on the size of the dose, and on the reacting power of the injected individual, there is an increased opsonic power, the positive phase, or else a continuation of the negative phase. The former is obtained with proper dosage, the latter with doses too large or too small. An increased opsonic power goes hand in hand with clinical improvement.

The present study has shown that the inoculations are not regularly followed by negative phases and, in some animal tests made, even the inoculation of very large doses of dead cultures failed to produce negative phases. The following table will suffice:

Dr. K. (case of Staphylococcus boils).	
Index on Dec. 22	0.65
" " " 23	0.65
" " " 24	0.67
Injected at noon and serum tested again, 12 hours later, Dec. 24	1.31
" 25	1.10

Similar results were obtained on three consecutive injections.

Clinical Results.

Our clinical experiences with the bacterial inoculation method of treatment have in some instances been very satisfactory and in other instances quite the opposite. In several cases of multiple boils due to staphylococcus,

the inoculations seem to have been of distinct value. It should, however, be emphasized that the clinical improvement was not paralleled by the opsonic index. Conversely also in some of the cases that did poorly, the opsonic index was regularly well above normal.

Altogether these observations indicate that the opsonic index is not necessarily a measure of the patient's immunity. This is not at all surprising to one who has followed the progress of imunity studies. When Gruber and Durham published their observations on agglutinins the phenomenon was at once hailed and interpreted by many as measuring the degree of immunity possessed by the patient. The same error was made when, some time later, the bacteriolytic substances were discovered. In both cases it was soon found that these were but accompaniments, of greater or less significance, of the complex phenomenon of immunity. It would certainly be surprising if the opsonic power were to be the real measure of the degree of immunity. When we consider how manifold are the defensive agencies which the animal organism possesses and how very complex they have shown themselves the more they are studied, we shall not marvel at the absence of parallelism between the clinical course of the disease and the opsonic index. We do not question that a study of the opsonic power may lead to a better understanding of certain phases of immunity. Apparently the opsonic curves do measure a certain fraction of the immunity reaction, and as such, possess distinct value. It is unlikely, however, that opsonic readings replace the ordinary clinical observations in measuring the effect of bacterial inoculations.

SUMMARY.—Measured in accordance with Wright's technique, one finds considerable variation in the opsonic power of serum from a number of apparently "normal" individuals.

In estimating the opsonic power the counting of only 50 leucocytes is insufficient. Good readings may, however, be obtained from 150 leucocytes.

For reasons not yet understood duplicate and triplicate tests made on the same serum, at the same time, and under apparently identical conditions, often yield widely divergent results.

Clinical results with bacterial inoculations do not always parallel the opsonic indices. Cases will do well with decreased opsonic index, and *vice versa.*

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